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## VARIANTS OF THE TULAREMIA MICROBE DUE TO EXTERNAL FACTORS

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There is no doubt that the various facts available to Soviet Science must be reviewed in the light of the new theories of Lysenko. A review of Gayskiy's work with living tularemia vaccine should be reviewed along these new lines of thought. The results which Gayskiy obtained conclusively proved the theories propounded by Michurin and Lysenko, that the influence of external factors brings about changes in an organism.

The tularemia microbe is considered the best specimen for the study of variants of microorganisms to disprove Weismannism in microbiology. Gayskiy's theories give no consideration whatsoever to the concept of genes, which in any case are not understood by anybody. The supporters of the gene "theory" contend that variants are a recombination of these elusive substances. They do not consider the effect of external factors and do not believe that the cell is the basis of heredity. Gayskiy, however, proved that changes which are brought about in a tularemia microbe cell can be and are inherited. Gayskiy proclaimed the indisputable fact that the cell is the transmitter of irreversible hereditary changes and that these changes can be controlled and are in no way accidental.

Care must be taken not to underestimate the value of Gayskiy's work for obtaining attenuated vaccine strains from virulent cultures of tularemia microbes. All of the data available to date confirms the value of Gayskiy's methods; strains which he prepared in 1943 are still effective and have high immunogenic properties. This obviously is a triumph for those who support the attenuation method. Experiences in past cases of mass vaccination of populations indicated that the vaccines used were not strong enough. This can be attributed to a selection of initially weak strains. Many of the strains became weak during



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their period of storage. Therefore, the only solution to this previously haphazard and ineffective method appears to be a method which utilizes the action of natural conditions toward the goal of producing controlled weakening of strains at vaccination strength.

The present article deals specifically with the most effective of the known attenuation methods. The treatment is general and confined to two aspects: (1) "irritation" by accidental factors, or (2) variation of environmental factors. All experimental results have disproved the anti-scientific thinking of some "scientist" who frown upon the suggestion that morphologic changes in microorganisms can be induced by variation of external factors.

In a long series of experiments which were started in late 1946, it was shown conclusively that theories promulgated by Michurin and Lysenko are correct. These tests were conducted with five virulent strains of tularemia cultures. Five methods, two of which were suggested by Gayskiy, were used to decrease the virulence of the strains. The first of these revolves around the variation of environment and the second involves the "irritation" of an immune serum.

Of the five strains used in the experiments three had very high virulence and two low virulence. One of the latter was satisfactory for use as a vaccine (administration to white mice in one million microbe doses produced death in 57.2 percent of the laboratory animals). It was shown that the virulence could be lowered by several methods, but that the time required varied.

Moreover, the various strains showed varying hereditary characteristics. Some strains which had particularly high virulence were made less virulent (some ten to 100 million times) over a period of 4-5 months, while in some cases where the original strains were not too virulent it was necessary to allow 7 months pass before the virulence was lowered to a point where the strains could be utilized for vaccination.

It was observed that changes in the tularemia strain occur immediately after they are isolated from the animal organism and are placed on artificial nutrient media. Variations in the external factors react slowly on the strains. Moreover, the methods used by the author to change the activity of the strains did not produce the same results in all cases. Simultaneous tests with the abovementioned five strains permitted determination of the fact that the method of radical changes in the conditions surrounding the media brought about the most rapid changes with respect to lowering the virulence of the strains.

The author also attempted to determine the effect of various factors on lowering the virulence of the strains. It was determined that specific immune serum has a very great effect, thus confirming the theories of certain scientists, such as Gayskiy, Arkrayt /Arkwright/, and others.

By storing the tularemia strains in immune serum it was possible to obtain strains which, when administered in white mice in doses up to one million microbes, produced death in 85 percent of the cases. However, strains which were attenuated for the same period of time produced death in only 80 percent of the white mice innoculated. Results obtained by using a strain which had been stored in a physiologic salt solution containing immune serum confirmed these conclusions. Moreover, this method failed to lower the virulence of the vaccine less than the one obtained from bullion media without serum. Thus, it was possible to determine that specific immune serum has no effect on lowering the virulence. The only advantage of using the serum is that the agglutination of microbes on a liquid medium greatly simplifies the handling of the microbes during the attenuation process.

Some attempts were made to study the effect of repeated selective cultures on lowering the virulence of tularemia strains. Some microbiologists claim that upon isolation from an animal, the saprophytic strains appear as a numerical majority due to the fact that the virulent strains are less suited for existence outside of the animal and die. They further contend that external factors which bring about a change in the metabolism of the microorganism have no significance and that these changes cannot be inherited.

A special series of experiments was conducted with a particularly virulent tularemia strain which, when administered in small doses, produced death in all the white mice inoculated. Attenuation was conducted by two methods but with the same time interval. Two different strains were obtained. One of the strains (by selection) produced death in 54 percent of the white mice when administered in doses up to one million microbes. The other strain which was obtained after it was subjected to the action of external factors produced deaths in only 20 percent of the white mice which were innoculated. Thus, it can be concluded that external factors which produce a change in the metabolism of the microbe have a very significant effect in the variation of the microorganism.

Experiments with five tularemia strains, prepared by various methods of attenuation, produced 12 different strains. In the case of nine of these strains it was possible to lower their virulence to a point where they were acceptable for vaccinations. Six of the strains were tested on laboratory animals to determine their immunogenic properties. Vaccines produced from these six strains were tried on volunteers. It was possible to determine that four of the above-mentioned strains did not attain the qualities of Gayskiy's strains which today are used in the manufacture of live antitularemia vaccines. On the other hand, some of these strains proved themselves to possess low immunogenic properties. It can be assumed that the use of a method which acts strongly on varying the cultural methods of the strains, does not affect the antigenic properties. If greater care is exercised it may be possible to obtain effective strains, which would retain their immunogenic properties for long periods and at the same time be satisfactory for use in innoculations.

Finally, an attempt was made to determine the relationship between the immunogenicity and virulence of the strains which were obtained by the various methods. Gayskiy and others seem to believe that strains which lose their virulence also lose their immunogenic properties. We have found data that contradicts Gayskiy's claims. Research conducted along the lines established by Michurin and Lysenko fully confirmed these theories.

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